Protective effects of oxymatrine against lipopolysaccharide/D-galactosamine-induced acute liver failure through oxidative damage, via activation of Nrf2/HO-1 and modulation of inflammatory TLR4-signaling pathways

JIAN XU[1], CHENGMIN LI[1], ZIWEI LI[2], CHAN YANG[1], LAN LEI[1], WEI REN[1], YAN SU[1] and CHUNPING CHEN[1]

Departments of [1]Infectious Disease and [2]Clinical Laboratory, Fuling Center Hospital of Chongqing, Chongqing 408008, P.R. China

Received October 25, 2016; Accepted June 13, 2017

DOI: 10.3892/mmr.2017.8060

Abstract. Oxymatrine has a variety of pharmacological functions, including anti-viral, anti-liver fibrotic, anti-cancer, anti-bacterial, anti-epidemic, analgesic, anti-allergy and anti-inflammatory properties. The present study aimed to investigate the protective effects of oxymatrine against lipopolysaccharide (LPS)/D-galactosamine (D-GalN)-induced acute liver failure and the associated underlying mechanisms. Mice were administrated 4 mg/kg LPS and 600 mg/kg D-GalN. Then, mice in the Oxymatrine group were treated with 120 mg/kg of oxymatrine for 4 weeks. Oxymatrine treatment increased survival rate, decreased plasma aspartate transaminase and alanine aminotransferase activity, increased superoxide dismutase and glutathione peroxidase and decreased malondialdehyde, tumor necrosis factor- and myeloperoxidase activities in mice with LPS/D-GalN-induced liver failure. Furthermore, Oxymatrine activated nuclear factor erythroid 2-related factor (Nrf) 2 and heme oxygenase (HO)-1 protein expression, and suppressed Toll like receptor (TLR)4, myeloid differentiation primary response 88 and nuclear factor-κB protein expression in mice LPS/D-GalN mice. Overall, the present study suggests that oxymatrine effectively attenuates LPS/D-GalN-induced acute liver failure by oxidative damage via activation of Nrf2/HO-1 and modulation of TLR4-dependent inflammatory signaling pathways.

Correspondence to: Dr Jian Xu, Department of Infectious Disease, Fuling Center Hospital of Chongqing, 2 Gaosuntang Avenue, Chongqing 408008, P.R. China
E-mail: dhxt1590026@126.com

Key words: oxymatrine, acute liver failure, nuclear factor erythroid 2-related factor 2, heme oxygenase 1, Toll like receptor 4

Introduction

Acute liver failure (ALF) is a type of severe liver disease which frequently occurs in patients with no medical history of liver disease, characterized by sudden apoptosis of liver cells over a short period, rapid deterioration of liver function and complications including hepatic encephalopathy and coagulation disorders (1). Due to high incidence, rapid development, severe illness, poor prognosis and high mortality rate, acute liver failure seriously endangers the life of patients and has become a challenge to clinicians (2). ALF exhibits a complex pathogenesis, and is a complicated pathophysiological process involving numerous factors. Endotoxemia and special liver toxic substances are important factors that lead to the incidence and development of liver failure (1). It is believed that lipopolysaccharide (LPS)/D-galactosamine (D-GalN) may lead to the injury of liver cells in mice by various mechanisms including liver cell apoptosis, generation of free radicals and lipid peroxidation (3). Research regarding the role of hepatocyte apoptosis and oxidative stress in the pathogenesis of ALF is of primary concern (4). It has previously been demonstrated that hepatic tissues of patients with ALF generate increased reactive oxygen species (ROS), resulting in an oxidation-reduction imbalance and thereby oxidative stress injury, which results in the apoptosis of liver cells by a mitochondrial pathway. Therefore, antioxidants and anti-apoptotic therapeutics are necessary in the treatment of ALF (5). Excluding liver transplantation, there is no effective therapeutic strategy in the treatment of ALF (6). Therefore, early therapeutic intervention in ALF has high research value and clinical significance.

A previous study suggested that transcription factor nuclear factor erythroid 2-related factor (Nrf) 2 is a key factor in the regulation of numerous antioxidants, which may maintain the balance of oxidation and reduction, inhibit apoptosis and protect against inflammation (7). The expression of Nrf2 is decreased under normal circumstances, very few stable Nrf2 units translocate into the nucleus and bind to antioxidant response elements (ARE) (8). However, the balance is disturbed under oxidative stress; Nrf2 escapes the Keap1-mediated ubiquitin degradation pathway and rapidly

translocates into the nucleus, binds to ARE to initiate the transcription of phase II detoxifying enzymes and various antioxidant enzyme genes downstream of ARE, thus resisting external harmful stimuli. Target genes in Nrf2 include heme oxygenase (HO-1), superoxide dismutase (SOD), dependent coenzyme/II oxidoreductase-1 and glutamylcysteine synthase. Deficiency or activation disorders of Nrf2 may increase the sensitivity of stimuli, leading to cell dysfunction and apoptosis, prolonged inflammatory repair and other pathological alterations (7,9).

Intestinal endotoxemia is important in liver failure. The pathophysiological process of endotoxin-induced liver injury involves a variety of signaling molecules and signal transduction pathways, of which the LPS/Toll like receptor (TLR)4 signal transduction pathway exhibits a predominant role (10). TLR4/nuclear factor (NF)-κB are classical inflammatory signals, and high expression of TLR4/NF-κB is associated with liver injury. It has been indicated that therapeutic intervention of the expression of TLR4 and NF-κB may improve liver function to a certain extent.

Oxymatrine (Fig. 1), additionally termed matrine, is an alkaloid with a tetracyclicquinolizidine structure extracted from *Sophora alopecuroides* L. (11), which belongs to the Ningxia herbal medicine Sophora. With anti-inflammatory, hepatoprotective and anti-neoplastic functions, Oxymatrine is effective in the treatment of cardiovascular disease (12). As a drug with various pharmacological effects, Oxymatrine has been applied to the treatment of hepatitis B and liver fibrosis, in addition to preventing chronic kidney disease development into renal interstitial fibrosis in patients, with low rates of adverse reaction (11,13). Therefore, the specific purpose of the present study was to investigate the protective effects of oxymatrine against LPS/D-GalN-induced acute liver failure and potential mechanisms in the mouse model.

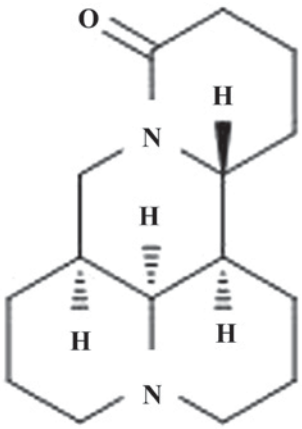

Figure 1. Chemical structure of Oxymatrine.

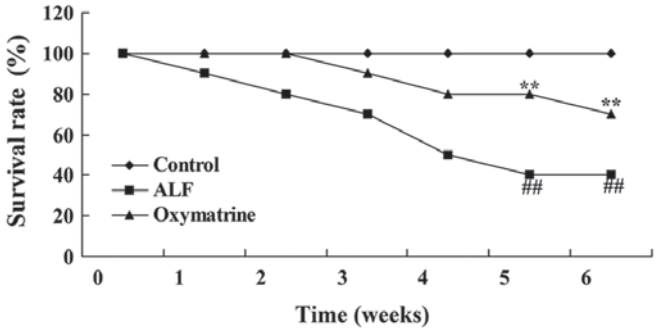

Figure 2. Oxymatrine increases survival rate in ALF mice. Survival rate of mice was measured in control, ALF model and Oxymatrine treated groups. ##P<0.01 vs. control group; **P<0.01 vs. ALF model group. ALF, acute liver failure; ALF model group, lipopolysaccharide/D-galactosamine-induced ALF model group; Control, control group; Oxymatrine, 120 mg/kg Oxymatrine group.

Materials and methods

Animals and experimental protocol. Male, C57BL/6 mice (weight, 20-22 g; age, 6 weeks old) were obtained from the Center of Experimental Animals of Chongqing University (Chongqing, China) and were housed under standard conditions (temperature, 22±2˚C; humidity, 55±5%, 12-h light/dark cycle) with free access to food and water. All animal experiments carried out in the present study were approved by the Care and Use of Laboratory Animals of Fuling Center Hospital of Chongqing (Chongqing, China). Mice were randomly assigned to normal, ALF model or Oxymatrine groups. In the ALF model and Oxymatrine groups, mice were administrated 4 mg/kg LPS and 600 mg/kg D-GalN (intraperitoneal injection) for 24 h. Then, mice of the Oxymatrine group were treated with 120 mg/kg/day oxymatrine for 4 weeks. Mice in the normal and ALF model groups were treated with normal saline.

Blood clinical analyses. Following treatment with Oxymatrine, blood samples were collected and centrifuged at 2,000 x g for 10 min at 4˚C. The serum was then collected and used to measure aspartate transaminase (AST) and alanine aminotransferase (ALT) levels using ELISA kits (C010-2 and C009-2 respectively; Nanjing Jiancheng Bioengineering Institute, Nanjing, China).

Analysis of oxidative stress and inflammation. Following treatment with Oxymatrine, the tissue samples were homogenized in lysis buffer (Beyotime Institute of Biotechnology, Jiangsu, China) and used to analyze superoxide dismutase (SOD), glutathione peroxidase (GSH-Px), malondialdehyde (MDA), tumor necrosis factor (TNF)-α and myeloperoxidase (MPO) activities using ELISA kits (A001-1-1, A005, A003-1, H052 and A044 respectively; Nanjing, Jiancheng Bioengineering Institute).

Western blot analysis. Following treatment with Oxymatrine, the tissues samples were homogenized in radioimmunoprecipitation assay buffer (Beyotime Institute of Biotechnology), and the protein concentrations were determined using a BCA protein assay reagent (Beyotime Institute of Biotechnology). A total of 50-60 μg proteins were separated on 10% SDS-PAGE gel and transferred onto polyvinylidene membranes (Thermo Fisher Scientific, Inc., Waltham, MA, USA). Following blocking with 5% skim milk for 1 h at 37˚C, the membranes were incubated with primary antibodies against Nrf2 (1:500; cat. no. sc-81342;), HO-1 (1:500; cat. no. sc-136256) TLR4

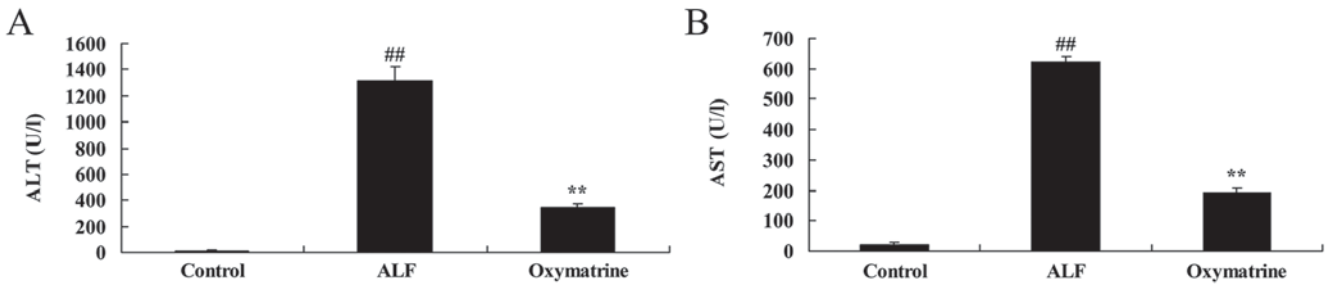

Figure 3. Oxymatrine decreases plasma AST and ALT activities in ALF mice. Oxymatrine decreased plasma (A) ALT and (B) AST activity in ALF mice. ##P<0.01 vs. control group; **P<0.01 vs. ALF model group. AST, aspartate transaminase; ALT, alanine aminotransferase; ALF, acute liver failure; ALF model group, lipopolysaccharide/D-galactosamine-induced ALF model group; Control, control group; Oxymatrine, 120 mg/kg Oxymatrine group.

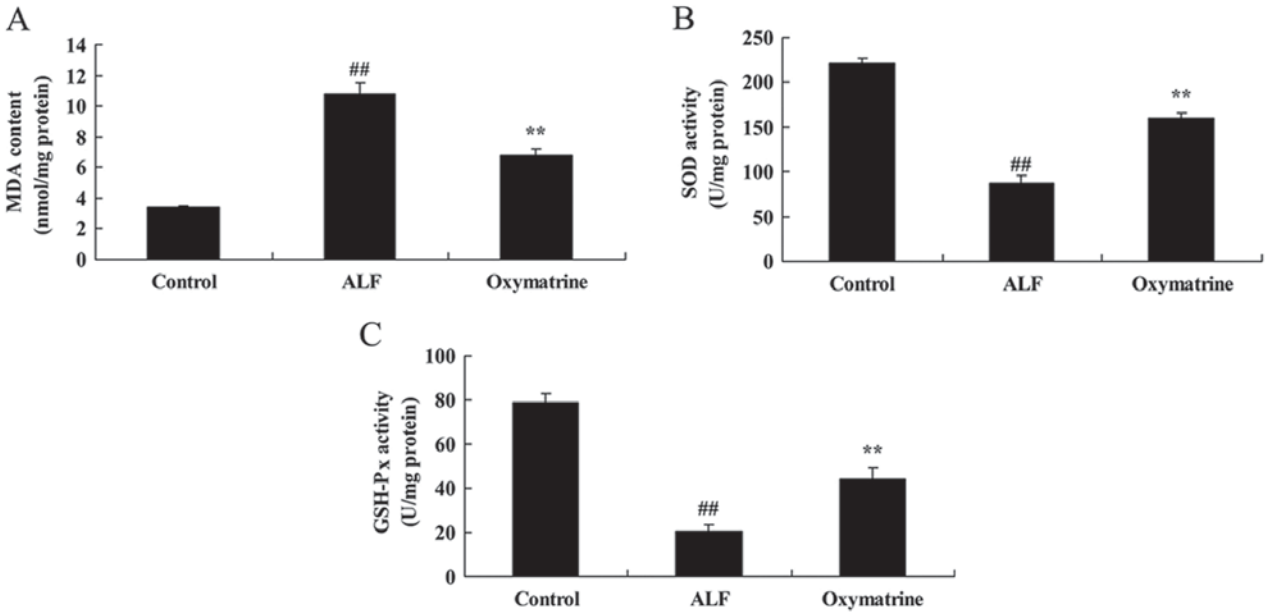

Figure 4. Effects of Oxymatrine on plasma MDA, SOD and GSH-Px activities in ALF mice. Oxymatrine decreased plasma (A) MDA and increased (B) SOD and (C) GSH-Px activities in ALF mice. ##P<0.01 vs. control group; **P<0.01 vs. ALF model group. MDA, malondialdehyde; SOD, superoxide dismutase; GSH-Px, glutathione peroxidase; ALF, acute liver failure; ALF model group, lipopolysaccharide/D-galactosamine-induced ALF model group; Control, control group; Oxymatrine, 120 mg/kg Oxymatrine group.

(1:300; cat. no. sc-293072), myeloid differentiation primary response 88 (MyD88; sc-11356; 1:300), NF-κB (1:300; cat. no. sc-136970) and GAPDH (1:300; cat. no. sc-59540) all obtained from Santa Cruz Biotechnology, Inc. (Dallas, TX, USA) at 4˚C overnight. Following washing with TBST 3 times, the membranes were probed with a horseradish peroxidase-conjugated secondary antibody (1:5,000; Cell Signaling Technology, Inc., Danvers, MA USA) at room temperature for 2 h. Protein was developed with the ECL Plus Western Blotting Detection system (GE Healthcare Life Sciences, Little Chalfont, UK) and analyzed using Image_Lab version 3.0 (Bio-Rad Laboratories, Inc., Hercules, CA, USA).

Statistical analysis. Data are presented as the mean ± standard deviation using SPSS version 19.0 (IBM Corp., Armonk, NY, USA). Comparison among groups was determined by one-way analysis of variance followed by the Tukey post hoc test. P<0.05 was considered to indicate a statistically significant difference.

Results

Oxymatrine increases survival rate in ALF mice. In LPS/D-GalN -induced ALF mice, survival rate was 40% which was a decreased value compared with normal control group. Treatment with Oxymatrine significantly increased survival rate of LPS/D-GalN-induced ALF mice, compared with the LPS/D-GalN-induced ALF model group (Fig. 2).

Oxymatrine decreases plasma AST and ALT serum levels in ALF mice. Conversely, AST and ALT serum levels of ALF model mice were increased compared with normal control group. Treatment with Oxymatrine significantly reduced AST and ALT serum levels in ALF mice, compared with ALF model group (Fig. 3).

Oxymatrine exhibits varied effects on SOD, GSH-Px and MDA activities in ALF mice. It was observed that there was a significant decrease in SOD and GSH-Px, and an increase in MDA activities in the ALF model group, compared with

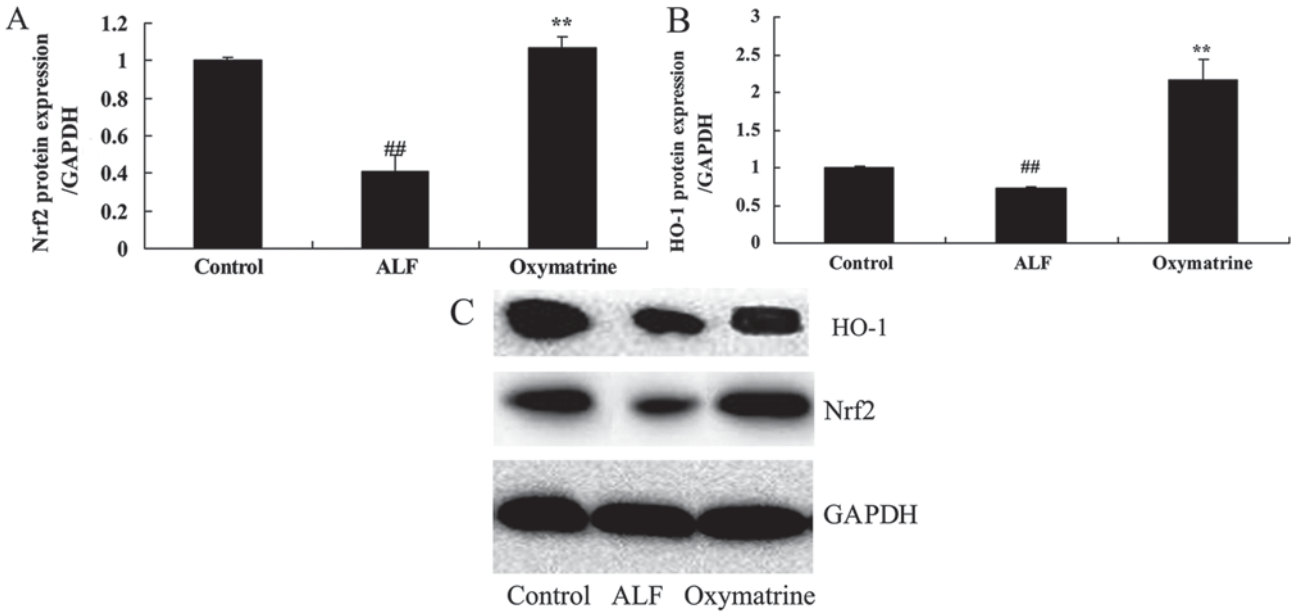

Figure 5. Oxymatrine increases Nrf2 and HO-1 protein expression in ALF mice. Oxymatrine activated Nrf2 and HO-1 protein expression demonstrated by (A and B) quantitative analysis and (C) representative image of western blotting in ALF mice. ##P<0.01 vs. control group; **P<0.01 vs. ALF model group. Nrf 2, nuclear factor erythroid 2-related factor 2; ALF, acute liver failure; ALF model group, lipopolysaccharide/D-galactosamine-induced ALF model group; Control, control group; Oxymatrine, 120 mg/kg Oxymatrine group; HO-1, heme oxygenase.

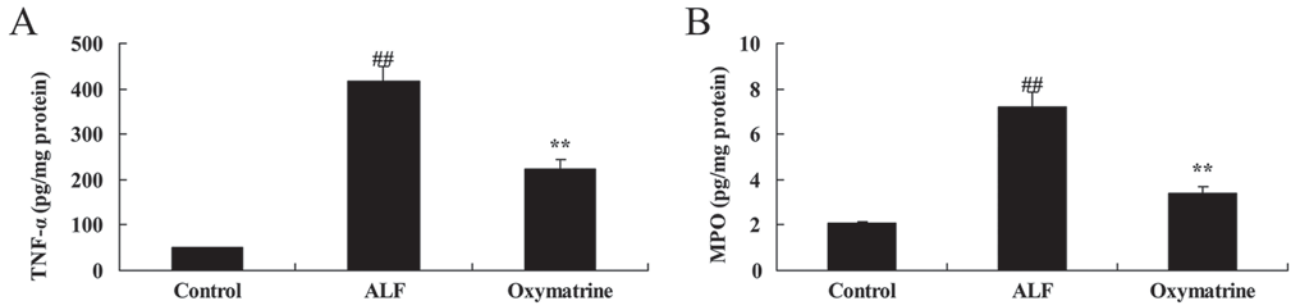

Figure 6. Oxymatrine suppresses plasma TNF-α and MPO activities in ALF mice. Oxymatrine decreased plasma (A) TNF-α and (B) MPO activities in ALF mice. ##P<0.01 vs. control group; **P<0.01 vs. ALF model group. TNF-α. tumor necrosis factor-α; MPO, myeloperoxidase; ALF, acute liver failure; ALF model group, lipopolysaccharide/D-galactosamine-induced ALF model group; Control, control group; Oxymatrine, 120 mg/kg Oxymatrine group.

normal control group. Pre-treatment with Oxymatrine significantly increased SOD and GSH-Px activities and decreased MDA activities in ALF mice, compared with the ALF model group (Fig. 4).

Oxymatrine activates Nrf2 and HO-1 protein expression in ALF mice. To explore the potential effects of Oxymatrine on ALF mice, Nrf2 and HO-1 protein expression was measured using western blot analysis. The results of the western blot analysis demonstrated that Nrf2 and HO-1 protein expression of ALF mice was downregulated in ALF mice, compared with normal control group. Oxymatrine significantly increased Nrf2 and HO-1 protein expression in ALF mice, compared with ALF model group (Fig. 5).

Oxymatrine decreases plasma TNF-α and MPO levels in ALF mice. The results demonstrated that TNF-α and MPO levels of the ALF model group were upregulated, compared with normal control group. Treatment with Oxymatrine significantly decreased TNF-α and MPO levels in ALF mice, compared with ALF model group (Fig. 6).

Oxymatrine suppresses TLR4, MyD88 and NF-κB protein expression in ALF mice. The present study next sought to investigate the role of TLR4, MyD88 and NF-κB. Protein expression was measured using Western blot analysis. As presented in Fig. 7, TLR4, MyD88 and NF-κB protein expression in ALF mice was increased, compared with normal control group. Oxymatrine significantly suppressed TLR4, MyD88 and NF-κB protein expression in ALF mice, compared with ALF model group (Fig. 7).

Discussion

The incidence of liver disease is high in China due to hepatitis B and C viral infections. According to the report of the World Health Organization, ~2 billion people are infected with hepatitis B worldwide and 350 million of these cases

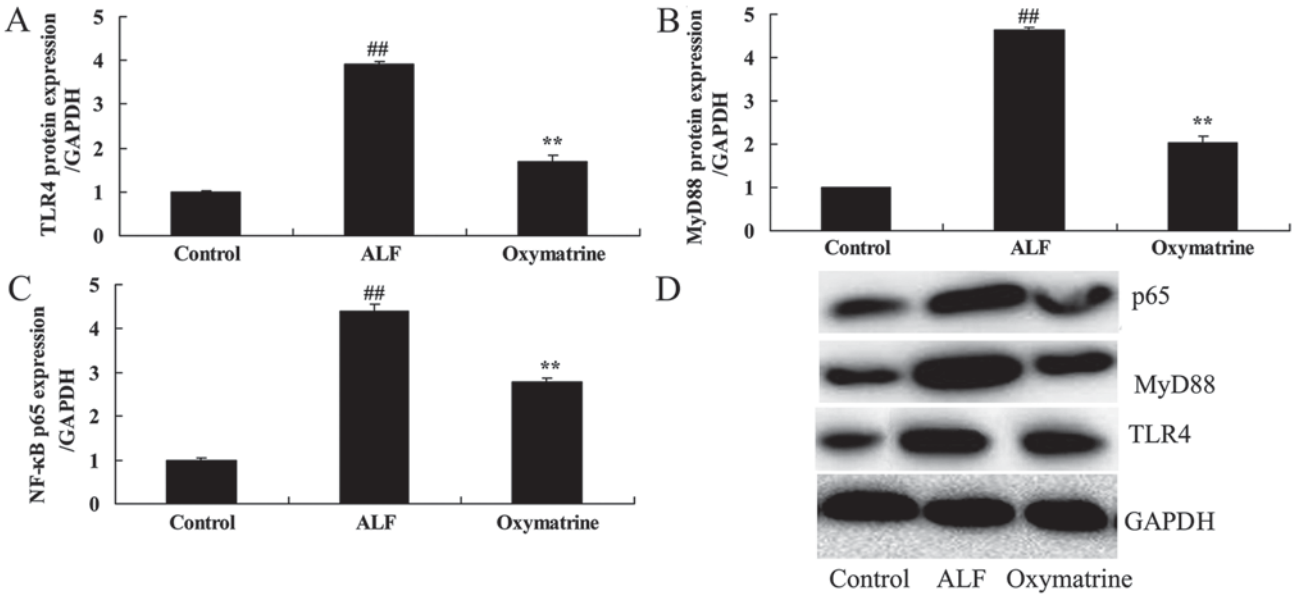

Figure 7. Oxymatrine suppresses TLR4, MyD88 and NF-κB protein expression in ALF mice. Oxymatrine suppressed TLR4, MyD88 and NF-κB protein expression as demonstrated by (A-C) quantitative analysis and (D) representative image of western blotting in ALF mice. ##P<0.01 vs. control group; **P<0.01 vs. ALF model group. TLR4, Toll like receptor 4; MyD88, myeloid differentiation primary response 88; NF-κB, nuclear factor-κB; ALF, acute liver failure; ALF model group, lipopolysaccharide/D-galactosamine-induced ALF model group; Control, control group; Oxymatrine, 120 mg/kg Oxymatrine group.

are chronic infections (3). In addition, ~1 million fatal cases of liver cirrhosis, liver failure and hepatocellular carcinoma are induced by hepatitis B every year (14). Hepatic cirrhosis accounts for 10-20% of all liver diseases based on pathological features and progression of disease (15). Liver failure may be divided into four categories, including acute liver failure, sub-acute liver failure, acute-on-chronic liver failure and chronic liver failure, each of which account for ~0.25-5% of advanced liver diseases, however the mortality rate may be up to 60-80% (2,15). Chronic or acute/subacute liver failure frequently occurs in China, accounting for >90% of all liver diseases (16). The present study firstly observed that Oxymatrine effectively increased the survival rate and reduced AST and ALT activities in LPS/ D-GalN-induced ALF mice. Li *et al* (17) reported that the effects of oxymatrine prevents against arsenic trioxide-induced liver injury.

The body has developed a set of complex systems in response to the damage that may result from oxidative stress, one of which is ARE (18). ARE is a cis-acting element located upstream of phase II detoxifying enzymes and antioxidant protein/enzyme genes (19). It has previously been demonstrated that Nrf2 is the activator of ARE and the key transcription factor which regulates the oxidative stress response, the sensor for toxic substances and exogenous oxidative stress, and is closely associated with incidence and development of inflammation, respiratory system disease, malignant tumors, precancerous lesions and cardiovascular diseases (8). Under normal circumstances, Nrf2 is in an inactive state via binding to cytoplasmic specific receptor Keap-1 in the form of a heterologous dipolymer (19). It is activated by oxidative stress, uncouples from Keap1 and translocates into the nucleus to bind to ARE. The activated ARE then mediates target gene transcription, to increase the resistance of cells to oxidative stress. SOD, HO-1 and various other Nrf2 target genes are expressed in the liver. HO-1, additionally termed heat shock protein 32, is an endogenous antioxidant enzyme which is of current research interest (20). It has previously been demonstrated that HO-1 and metabolic products of heme may protect against oxidative stress, and the targeted activation of HO-1 may prevent injury to the liver (9,21). The results of the present study demonstrated that Oxymatrine significantly increased SOD and GSH-Px activities and decreased MDA activities in ALF mice through activating Nrf2/HO-1 expression. Jiang *et al* (22) demonstrated that Oxymatrine ameliorates renal ischemia-reperfusion injury via the Nrf2/HO-1 pathway.

TLR4 is one of the natural immune recognition receptors which is primarily used to recognize LPS and mediate transmembrane signal transduction. LPS activates Kupffer cells (KC) via a TLR-mediated signal transduction pathway, with a mechanism as follows: LPS and TLR4 interact with each other to transfer LPS into TLR4/lymphocyte antigen 96 (MD2) by LPS binding protein (LBP) and cluster of differentiation 14, and then LPS binds to TLR4/MD2 to induce the accumulation of TLR4, leading to the activation of intracellular MyD88 dependent pathway, and thereby promoting the expression and activation of NF-κB in cells, so as to stimulate cells to produce a variety of inflammatory mediators including TNF, IL-1, IL-6, nitric oxide, leukotrienes and thus resulting in the inflammatory reaction (23-25). This aids in the body's removal of endotoxins, however an excessive inflammatory reaction results in liver cell apoptosis and necrosis (26). It was demonstrated that Oxymatrine significantly suppressed TNF-α and MPO activities via the TLR4/MyD88/NF-κB-dependent inflammatory signaling pathways. Fan *et al* (27) reported that Oxymatrine protects rat brains against focal ischemia and downregulates TLR4, TLR2, MyD88 and NF-κB.

In conclusion, oxymatrine effectively attenuated LPS/ D-GalN-induced ALF through oxidative damage, by activation of Nrf2/HO-1 and suppression of TLR4-dependent inflammatory signaling pathways. Identification of the mechanism

underlying these effects of Oxymatrine may aid in the development of a novel therapeutic strategy and subsequent clinical application in the treatment of ALF in the future.

Acknowledgements

The present study was partially supported by Health and Family Planning Commission of Chongqing municipal, China (grant no. 2016ZDXM038).

References

1. Yonekawa C, Nakae H, Tajimi K and Asanuma Y: Effectiveness of combining plasma exchange and continuous hemodiafiltration in patients with postoperative liver failure. Artif Organs 29: 324-328, 2005.
2. Nalos M, Leverve X, Huang S, Weisbrodt L, Parkin R, Seppelt I, Ting I and Mclean A: Half-molar sodium lactate infusion improves cardiac performance in acute heart failure: A pilot randomised controlled clinical trial. Crit Care 18: R48, 2014.
3. Lee WM, Hynan LS, Rossaro L, Fontana RJ, Stravitz RT, Larson AM, Davern TJ II, Murray NG, McCashland T, Reisch JS, *et al*: Intravenous N-acetylcysteine improves transplant-free survival in early stage non-acetaminophen acute liver failure. Gastroenterology 137: 856-864, 864.e1, 2009.
4. Moniaux N, Song H, Darnaud M, Garbin K, Gigou M, Mitchell C, Samuel D, Jamot L, Amouyal P, Amouyal G, *et al*: Human hepatocarcinoma-intestine-pancreas/pancreatitis-associated protein cures fas-induced acute liver failure in mice by attenuating free-radical damage in injured livers. Hepatology 53: 618-627, 2011.
5. Shi M, Zhang Z, Xu R, Lin H, Fu J, Zou Z, Zhang A, Shi J, Chen L, Lv S, *et al*: Human mesenchymal stem cell transfusion is safe and improves liver function in acute-on-chronic liver failure patients. Stem Cells Transl Med 1: 725-731, 2012.
6. Larsen FS, Schmidt LE, Bernsmeier C, Rasmussen A, Isoniemi H, Patel VC, Triantafyllou E, Bernal W, Auzinger G, Shawcross D, *et al*: High-volume plasma exchange in patients with acute liver failure: An open randomised controlled trial. J Hepatol 64: 69-78, 2016.
7. Huang YJ, Chen P, Lee CY, Yang SY, Lin MT, Lee HS and Wu YM: Protection against acetaminophen-induced acute liver failure by omentum adipose tissue derived stem cells through the mediation of Nrf2 and cytochrome P450 expression. J Biomed Sci 23: 5, 2016.
8. Mobasher MA, González-Rodriguez A, Santamaría B, Ramos S, Martín MÁ, Goya L, Rada P, Letzig L, James LP, Cuadrado A, *et al*: Protein tyrosine phosphatase 1B modulates GSK3β/Nrf2 and IGFIR signaling pathways in acetaminophen-induced hepatotoxicity. Cell Death Dis 4: e626, 2013.
9. Wang CF, Wang ZY and Li JY: Dual protective role of HO-1 in transplanted liver grafts: A review of experimental and clinical studies. World J Gastroenterol 17: 3101-3108, 2011.
10. Xu CL, Hao YH, Lu YP, Tang ZS, Yang XC, Wu J, Zheng X, Wang BJ, Liu J and Yang DL: Upregulation of toll-like receptor 4 on T cells in PBMCs is associated with disease aggravation of HBV-related acute-on-chronic liver failure. J Huazhong Univ Sci Technolog Med Sci 35: 910-915, 2015.
11. Zhao HW, Zhang ZF, Chai X, Li GQ, Cui HR, Wang HB, Meng YK, Liu HM, Wang JB, Li RS, *et al*: Oxymatrine attenuates CCl4-induced hepatic fibrosis via modulation of TLR4-dependent inflammatory and TGF-β1 signaling pathways. Int Immunopharmacol 36: 249-255, 2016.
12. Zhang W, Zhang J, Liu YK, Liu J, Wang X, Xu Q, Wang Y, Xu X and Dai G: Cardioprotective effects of oxymatrine on isoproterenol-induced heart failure via regulation of DDAH/ADMA metabolism pathway in rats. Eur J Pharmacol 745: 29-35, 2014.
13. Fu L, Xu Y, Tu L, Huang H, Zhang Y, Chen Y, Tao L and Shen X: Oxymatrine inhibits aldosterone-induced rat cardiac fibroblast proliferation and differentiation by attenuating smad-2,-3 and -4 expression: An in vitro study. BMC Complement Altern Med 16: 241, 2016.
14. Qin G, Shao JG, Wang B, Shen Y, Zheng J, Liu XJ, Zhang YY, Liu YM, Qin Y and Wang LJ: Artificial liver support system improves short- and long-term outcomes of patients with HBV-associated acute-on-chronic liver failure: A single-center experience. Medicine (Baltimore) 93: e338, 2014.
15. Sterneck M, Settmacher U, Ganten T, Sarrazin C, Speidel N, Broering D, Heyne N, Paulus E, Mertens M and Fischer L: Improvement in gastrointestinal and health-related quality of life outcomes after conversion from mycophenolate mofetil to enteric-coated mycophenolate sodium in liver transplant recipients. Transplant Proc 46: 234-240, 2014.
16. Kribben A, Gerken G, Haag S, Herget-Rosenthal S, Treichel U, Betz C, Sarrazin C, Hoste E, Van Vlierberghe H, Escorsell A, *et al*: Effects of fractionated plasma separation and adsorption on survival in patients with acute-on-chronic liver failure. Gastroenterology 142: 782-789.e3, 2012.
17. Li L, Liu Q, Fan L, Xiao W, Zhao L, Wang Y, Ye W, Lan F, Jia B, Feng H, *et al*: Protective effects of oxymatrine against arsenic trioxide-induced liver injury. Oncotarget 8: 12792-12799, 2017.
18. Crespo I, Miguel BS, Laliena A, Álvarez M, Culebras JM, González-Gallego J and Tuñón MJ: Melatonin prevents the decreased activity of antioxidant enzymes and activates nuclear erythroid 2-related factor 2 signaling in an animal model of fulminant hepatic failure of viral origin. J Pineal Res 49: 193-200, 2010.
19. Xu W, Hellerbrand C, Köhler UA, Bugnon P, Kan YW, Werner S and Beyer TA: The Nrf2 transcription factor protects from toxin-induced liver injury and fibrosis. Lab Invest 88: 1068-1078, 2008.
20. FangFang, Li H, Qin T, Li M and Ma S: Thymol improves high-fat diet-induced cognitive deficits in mice via ameliorating brain insulin resistance and upregulating NRF2/HO-1 pathway. Metab Brain Dis 32: 385-393, 2017.
21. Chen X, Gong X, Jiang R, Wang B, Kuang G, Li K and Wan J: Resolvin D1 attenuates CCl4-induced acute liver injury involving up-regulation of HO-1 in mice. Immunopharmacol Immunotoxicol 38: 61-67, 2016.
22. Jiang G, Liu X, Wang M, Chen H, Chen Z and Qiu T: Oxymatrine ameliorates renal ischemia-reperfusion injury from oxidative stress through Nrf2/HO-1 pathway. Acta Cir Bras 30: 422-429, 2015.
23. Luo JX, Zhang Y, Hu XY, Chen G, Liu XY, Nie HM, Liu JL and Wen DC: Aqueous extract from *Aconitum carmichaelii* Debeaux reduces liver injury in rats via regulation of HMGB1/TLR4/NF-KappaB/caspase-3 and PCNA signaling pathways. J Ethnopharmacol 183: 187-192, 2016.
24. Liu H, Zhang W, Dong S, Song L, Zhao S, Wu C, Wang X, Liu F, Xie J, Wang J and Wang Y: Protective effects of sea buckthorn polysaccharide extracts against LPS/d-GalN-induced acute liver failure in mice via suppressing TLR4-NF-κB signaling. J Ethnopharmacol 176: 69-78, 2015.
25. Liang Q, Zhang J, Tang W, Geng Q, Xu X and Jiang W: Triptolide attenuates acute small-for-size liver graft injury in rats by inhibition of Toll-like receptor 4. Transplant Proc 46: 3303-3308, 2014.
26. Shah N, Montes de Oca M, Jover-Cobos M, Tanamoto K, Muroi M, Sugiyama K, Davies NA, Mookerjee RP, Dhar DK and Jalan R: Role of toll-like receptor 4 in mediating multiorgan dysfunction in mice with acetaminophen induced acute liver failure. Liver Transpl 19: 751-761, 2013.
27. Fan H, Li L, Zhang X, Liu Y, Yang C, Yang Y and Yin J: Oxymatrine downregulates TLR4, TLR2, MyD88, and NF-kappaB and protects rat brains against focal ischemia. Mediators Inflamm 2009: 704706, 2009.